Please add the following new claim:

20. (New) A population of hepatocytes and nonparenchymal cells, derived from hepatic cell lines comprising hepatocytes and nonparenchymal cells, associated with a matrix coated with at least one biologically active molecule that promotes cell adhesion, proliferation or survival.

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REMARKS

Claims 1-7 and 12-14 are pending in the application. The pending claims are rejected under 35 U.S.C. § 112 first and second paragraph. Claims 1, 2, 4-7 and 12 and 14 are rejected under 35 U.S.C. § 103(a). For reasons detailed below, the rejections should be withdrawn and the claims allowed to issue. Entry of the foregoing amendments is respectfully requested.

1. The Claims are Enabled

Claims 1-7, 12 and 14 are rejected under 35 U.S.C. § 112, first paragraph. The Examiner alleges that while the specification is enabling for the method of claim 1 wherein hepatocytes and nonparenchymal cells of the co-culture are obtained by perfusion of liver tissue with collagenase and for the population of matrix/hepatic cell clusters of claim 14 obtained by this method, the specification does not reasonably provide enablement for a method of providing a combination of hepatocytes and nonparenchymal cells for co-culturing as claimed, and for obtaining the population of matrix/hepatic cell clusters of claim 14 by another method.

The test for enablement is whether one reasonably skilled in the art could make and use the invention, without undue experimentation, from the disclosure in the patent

specification coupled with information known in the art at the time the patent application was filed. *U.S. v. Telectronics Inc.*, 857 F. 2d. 778, 8 USPQ 2d 1217. Furthermore, a patent need not teach, and preferably omits, what is well known in the art. *Hybridtech Inc., v. Monoclonal Antibodies, Inc.* 802 F 2d., 1367, 231 USPQ 81 (Fed. Cir. 1986).

Applicants have amended Claim 1 to indicate that the hepatocytes and non-parenchymal cells are derived from disaggragated liver tissue. In this regard the Examiner's, attention is directed to page 14, lines 3-22 of the specification, which discloses that hepatic and nonparenchymal cells can be isolated by a number of different methods, including techniques that are known to those of skill in the art. Such methods include disaggregation of liver tissue mechanically on enzymatically.

In addition, Claim 2 has been amended to indicate that the hepatocytes and non-parenchymal cells can be derived from established hepatic cell cultures. Support for the amendment to claim 2 can be found on page 14, line 22 through page 15, line 2 of the specification.

Based on the disclosure of specification, the pending claims are fully enabled for the entire scope of the recited subject matter. Therefore, the rejections under 35 U.S.C. §112, first paragraph, should be withdrawn.

2. The Claims are Definite

Claims 1-7, 12 and 14 are rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicants regard as the invention.

According to the Examiner, the claims are unclear by claim 1 not having clear antecedent basis for " said hepatocytes that retain hepatic function. Applicants have amended the claims as suggested by the Examiner.

In view of the foregoing amendments to the claims, the rejections under 35 U.S.C. §112, second paragraph, should be withdrawn.

3. The Claimed Invention is Not Obvious

Claims 1, 2, 4-7, 12 and 14 are rejected under 35 U.S.C. § 103(a) as being unpatentable over Mitaka et al. (Hepatology 1999; "Mitaka") in view of Naughton et al. (U.S. 5,624,840; "Naughton") and Vacanti et al. (U.S. 5,759,830; "Vacanti").

Mitaka is alleged to disclose obtaining hepatic cells and nonparenchymal cells from liver tissue and culturing the hepatic cells and nonparenchymal cells together for hepatic organoid reconstruction.

According to the Examiner, Naughton discloses growing stromal cells on a three-dimensional matrix such as made from nylon or polystyrene (col. 8, line 1) which may be coated with collagen (col. 8, line 8) to form a three-dimensional stromal matrix (col. 8, lines 30-40), and then growing hepatocytes on the stromal matrix to form tissue having liver function (col. 11, lines 54-57). The Examiner maintains that Vacanti discloses growing hepatocytes (col. 6, line 28) in a three-dimensional fibrous scaffold to form tissue having liver function for implanting (col. 5, line 35 to col. 6, line 62, and col. 12, lines 17-47). The fibers of the scaffold may be coated with collagen to enhance cell attachment (col. 10, lines 44-47), and epithelial cells may be attached to the scaffold in combination with the hepatocytes (col. 12, lines 25-27).

The Examiner asserts that it would have been obvious to carry out the culturing of hepatic cells and nonparenchymal cells together as disclosed by Mitaka on a three-dimensional matrix or scaffold as suggested by Naughton and Vacanti to obtain the function of the matrix or scaffold in producing tissue having liver function. Moreover, it would have been obvious to grow hepatocytes directly on the matrix without first forming stromal tissue since it is clear from Vacanti that stromal tissue can be omitted.

Claim 3, which requires that the matrix be in the form of polystyrene beads, is rejected under 35 U.S.C. § 103(a) as being unpatentable over the references as applied to claims 1, 2, 4-7, 12 and 14 above, and further in view of Matsui et al. (U.S. 5,298,615; "Matsui").The Examiner alleges that Matsui discloses that it is standard procedure to culture animal cells on microcarriers such as polystyrene beads coated with collagen (col. 2, lines 10-25).

Applicants respectfully disagree with the Examiner's rejection and submit that the claimed invention is not rendered obvious by the cited references using the objective standard for obviousness under 35 U.S.C. §103. As set forth in *Graham v. Deere*, a finding of obviousness under 35 U.S.C. §103 requires a determination of the scope and content of the prior art, the level of ordinary skill in the art, the differences between the claimed subject matter and the prior art, and whether the differences are such that the subject matter as a whole would have been obvious to one of ordinary skill in the art at the time the invention was made. *Graham v Deere, Inc.* 383 U.S. 1 (1966).

First, because the January 1999 publication date of Mitaka is within one year of the December 7, 1999 priority date of the present application and given the Declaration of Dr. George K. Michalopolulos and William C. Bowen, submitted herewith, stating that

they had derived data demonstrating the co-culturing of hepatic cells and nonparenchymal cells prior to the publication date of Mitaka, the reference of Mitaka is unavailable as a prior art reference.

Thus, in the present instance, the proper inquiry is whether Naughton, Vacanti and/or Matsui suggest the claimed methods for generating a hepatic cell culture comprising co-culturing hepatocytes and nonparenchymal cells, in the presence of growth factors and a matrix coated with a molecule that promotes cell adhesion. Clearly the answer to this question is no. Naughton merely discloses growing stromal cells on a three dimensional matrix followed by the growing of hepatocytes on the stromal matrix; Vacanti discloses growing hepatocytes in a three-dimensional fibrous scaffold to form tissue having liver function for implanting; and Matsui discloses that it was known to culture animal cells on microcarriers such as polystyrene beads coated with collagen. Each of the cited references, either alone or in combination, fail to disclose, or suggest, the benefit derived from co-culturing hepatocytes and non-parenchymal cells together on a matrix in the presence of growth factors.

Applicants assert that in the present instance, the Examiner has failed to produce any reference that teaches or suggests the co-culturing of hepatocytes and parenchymal cells, much less, the co-culturing of such cells on a matrix coated with a factor that promotes adhesion in the presence of growth factors. Therefore, the claimed invention is not obvious, and the rejections under 35 U.S.C. §103 should be withdrawn.

CONCLUSION

Entry of the foregoing amendments and remarks into the file of the above-identified application is respectfully requested. Applicants believe that the invention described and defined by the amended claims is patentable over the rejections of the Examiner. Withdrawal of all rejections and reconsideration of the amended claims is requested. An early allowance is earnestly sought.

Respectfully submitted,

Dated: December 16, 2002

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APPENDIX

IN THE CLAIMS:

Please amend the claims as follows:

1. (Twice amended) A method for generating a hepatic cell culture comprising co-culturing hepatocytes and nonparenchymal cells derived from disaggregated liver tissue, in the presence of growth factors and a matrix coated with at least one biologically active molecule that promotes cell adhesion, proliferation or survival under conditions sufficient to allow for the proliferation of said hepatocytes while retaining hepatic function of said hepatocytes.

2. (Amended) [The method of claim 1 wherein the hepatocytes and nonparenchymal cells are derived from a liver tissue sample] A method for generating a hepatic cell culture comprising co-culturing established hepatic cell lines comprising hepatocytes and non-parenchymal cells, in the presence of growth factors and a matrix coated with at least one biologically active molecule that promotes cell adhesion, proliferation or survival under conditions sufficient to allow for the proliferation of said hepatocytes while retaining hepatic function of said hepatocytes.

14. (Twice amended) A population of hepatocytes and nonparenchymal cells, derived from disaggregated liver tissue, associated with a matrix coated with at least one biologically active molecule that promotes cell adhesion, proliferation or survival.

Please add the following new claim:

--20. (New) A population of hepatocytes and nonparenchymal cells, derived from hepatic cell lines comprising hepatocytes and non-parenchymal cells, associated with a matrix coated with at least one biologically active molecule that promotes cell adhesion, proliferation or survival.--